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TOWNSEND and TOWNSEND and CREW LLP

By: Malwida Dagit

PATENT
Attorney Docket No.: 020130-000112US
Client Ref. No.: MJP0011(US)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

WANG et al.

Application No.: 10/821,583

Filed: April 9, 2004

For: IMPROVED NUCLEIC ACID MODIFYING ENZYMES

Customer No.: 20350

Confirmation No. 1973

Examiner: Richard G. Hutson

Technology Center/Art Unit: 1652

DECLARATION UNDER 37 C.F.R. § 1.132 by Yan Wang, Ph.D.

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

1. I, Yan Wang, am one of the inventors of the subject matter claimed in the above-referenced application. I am presently employed by Bio-Rad Laboratories, Inc as an R&D Manager in the Department of Reagent Development of the Gene Expression Division. My responsibilities include leading the Reagent Development R&D group in developing amplification reagents. I have worked in the area of nucleic acid amplification and DNA polymerase structure and function for over 15 years. A copy of my CV is attached

2. This Declaration provides additional data to support that a sequence non-specific double-stranded nucleic acid binding protein, such as Sso7d, enhances processivity of multiple, diverse polymerases when fused to the polymerases.

3. The experiments to obtain the data shown here were performed under my supervision. Comparative data are provided in this Declaration showing the effects of polymerase fusion to Sso7d on the processivity of the following polymerases:

Taq polymerase;

Thermus brockianus polymerase (*Tbr*) polymerase;

Tbr polymerase Stoffel fragment (which lacks the 5' to 3' exonuclease domain); and

the Klenow fragment of *Escherichia coli* DNA polymerase I (Pol I).

Tbr and *Taq* polymerases are not error-correcting, as they lack a functional 3' to 5' exonuclease domain. However, they retain the same overall structure as *E. coli* Pol I, which does have error-correcting activity.

4. Recombinant polymerase Sso7d fusion proteins were constructed and expressed in *E. coli* using well known technology. Recombinant polymerases without the Sso7d protein for comparative studies of processivity were also expressed in *E. coli*.

5. Processivity was measured by determining the number of nucleotides incorporated during a single binding event of the polymerase to a primed template. Briefly, a 5' FAM-labeled primer (34 nt long) was annealed to circular or linearized ssM13mp18 DNA to form the primed template. The primed template was mixed with the DNA polymerase of interest at a molar ratio of approximately 4000:1 (primed DNA: DNA polymerase) in the presence of standard PCR buffer (free of Mg++) and 200 μM of each dNTPs. $MgCl_2$ was added to a final concentration of 2 mM to initiate DNA synthesis. At various times after initiation, samples were quenched with sequencing loading dye and analyzed on a sequencing gel. The median product length, which is defined as the product length above or below which there are equal amounts of products, was determined based on integration of all detectable product peaks. At a polymerase concentration for which the median product length does not change with time, the median

product length corresponds to the processivity of the enzyme. The ranges presented in the Table below represent the range of values obtained in several repeats of the assay.

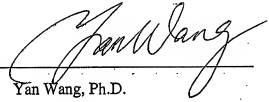
Polymerase	Processivity
Taq	13-15 nt
Exo-Sso-*Taq* (Sso7d inserted in the middle of *Taq* polymerase between the Exo domain and the Stoffel domain)	59-69
Thermus brockianus (*Tbr*) polymerase	11-13 nt
Sso7d fused to the N-terminus of *Tbr* polymerase	39-60 nt
Stoffel fragment of *Tbr* polymerase Stoffel lacks	2-4 nt (based on Stoffel result and a different clone of *Tbr* pol)
Sso7d fused to the N-terminus of the Stoffel	23-24 nt
Klenow	4-7 nt
Sso7d-Klenow	> 9-12 nt

6. The results show that Sso7d enhanced processivity of all of the polymerases analyzed. Although the enhancement of processivity of Klenow was not as great as the enhancement of processivity of the other polymerases analyzed here, it was nonetheless significant. The reduced extent of processivity enhancement with Klenow may relate to the fact that Sso7d is a thermal stable protein and may have a higher binding affinity for DNA at higher temperature, whereas Klenow and Klenow fusion were analyzed at temperatures below 40°C.

7. Based on our observations of the widespread enhancement effects of Sso7d on the processivity of multiple, diverse polymerases, including *Pfu* polymerase, variant *Pyrococcus* polymerases; *Taq* polymerase, *Tbr* polymerase, and Klenow, as well as fragments of *Taq* and *Tbr* polymerases, one of skill could reasonably expect that Sso7d or a related family member, when fused to a polymerase of interest, would enhance processivity of that polymerase as well.

All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

Date: 8/18/2009

By: 

Yan Wang, Ph.D.

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YAN WANG, Ph.D.

EXPERIENCE:

2008-Present *R&D Manager, Dept. of Reagent Development, Gene Expression Division, Bio Rad Lab*
Lead the Reagent Development R&D group in developing products and technologies for gene expression analyses through enzyme improvement and formulation optimization.

2003-2007 *Principle Scientist/R&D Manager, Dept. of System Integration, GXD. Bio Rad Lab.(Employer change due to acquisition.)*
Lead the Reagent Development R&D group in developing amplification reagent and in enzyme improvement.

2003-2004 *Principle Scientist, Department of R&D, MJ Bioworks, Inc.*
Lead two R&D groups, Reagents Development and Instrument Analyses, of 10 people (scientists and research associates). Oversaw the planning and execution of a number of reagents development projects. Ensured support to the instrument development effort. Coordinated collaborations with external R&D groups.

2001-2003 *Senior Scientist, Department of R&D, MJ Bioworks, Inc.*
Lead the Reagents Development R&D team of 5 people (scientist and research associates). Oversaw the development and successful launch of several commercial products.

1998-2001 *Associate Scientist/Research Scientist, Department of R&D, MJ Bioworks, Inc.*
Carried out research project developing new technologies that improve the in vitro performance of DNA polymerases. Independently conceived and validated the Sso7d technology as a novel strategy to improve the processivity of DNA polymerase. The development of this idea ensured a strong IP position of MJ Bioworks, changed the R&D direction of the company, and eventually enabled the launch of several commercial products in the subsequent years.

1993-1998 *Postdoctoral fellow, Christine Guthrie lab, Department of Biochmistry, UCSF*
Carried out research project investigating the structure/function relationship of an RNA dependent ATPase and putative RNA helicase, Prp16, which is involved in pre-mRNA splicing in yeast. Have applied combined approach of Genetics, molecular biology and biochemistry in achieving the research objective.

1986-1992 *Graduate student, Peter von Hippel lab, Institute of Molecular Biology, Univ. of Oregon.*
Completed a doctoral thesis project that studies the mechanism of rho-dependent transcription termination using combined approaches of biochemical and biophysical analyses. Systematically studied the interactions between purified protein and a large number of oligomer RNAs with designed sequences to elucidate the effects of nucleotide sequence on protein-RNA interactions.

EDUCATION:

1986-1992 Ph.D. in Biochemistry, University of Oregon.
1982-1986 B.S. in chemistry, Beijing University, P.R.C.

AWARDS:

1996-1998 The American Cancer Society (ACS) Postdoctoral Fellowship.

1993-1996 The Damon Runyon-Walter Wintchel Cancer Research Postdoctoral Fellowship.
1991-1992 A Research Fellow of the Institute of Molecular Biology, University of Oregon.

PUBLICATIONS:

1. Yan Wang, Dennis Prosen, Li Mei, John Sullivan, Michael Finney, and Peter Vander Horn (2004) A novel strategy to engineer DNA polymerase for enhanced processivity and improved performance *in vitro*. *Nucleic Acids Research*, **32**, 1197-1207. (http://nar.oupjournals.org/cgi/content/full/32/3/1197)
2. Yan Wang and Christine Guthrie (1998) PRP16, a DEAH-box RNA helicase, is recruited to the spliceosome primarily via its nonconserved N-terminal domain. *RNA* **4**, 1216-1229.
3. Yan Wang, John Wagner, and Christine Guthrie, (1998) Prp16, A DEAH-box splicing factor, unwinds RNA duplexes in vitro. *Curr Biol.* **8**, 441-451
4. Johannes Geiselmann, Yan Wang, Steven E. Seifried and Peter H. von Hippel. (1993) A physical model of the translocation and helicase activities of *Escherichia coli* transcription termination protein Rho. *Proc. Natl. Acad. Sci. USA* **90**, 7754-7758.
5. Yan Wang and Peter H. von Hippel. (1992) E. coli transcription termination factor Rho. II. Binding of oligonucleotide cofactors. Journal of Biological Chemistry 268, 13947-13955.
6. Yan Wang and Peter H. von Hippel. (1992) E. coli transcription termination factor Rho. I. ATPase activation by oligonucleotide cofactors. Journal of Biological Chemistry 268, 13940-13946.
7. Steven E. Seifried, Yan Wang and Peter H. von Hippel. (1988) Fluorescent Modification of the Cysteine 202 residue of Escherichia coli termination factor Rho. Journal of Biological Chemistry 263, 13511-13514.

PATENTS:

Issued Patent:

US7,560,260, Vander Horn,P.B. and Wang,Y. Compositions with polymerase activity.
US7,541,170, Wang et al. Nucleic acid modifying enzymes
US7,445,898, Li,B. Xi.L, Wang,Y. Vander Horn, P.B. Quantitative amplification with a labeled probe and 3' to 5' exonuclease activity.
US7,163,790, Wang et al. Parallel polymorphism scoring by amplification and error correction.
US6,627,424, Wang,Y. Nucleic acid modifying enzymes